Controlling neuronal network oscillations with GABAergic inhibition

Heistek, T.S.

2010

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General introduction

Brain oscillations and cognitive behavior

Complex behavior and cognition in humans and animals require timed activity of networks of neurons in different brain areas. Groups of neurons that are involved in a task, fire synchronously and repeatedly. Depending on the behavior, synchronization of activity of these neurons occurs at particular rhythms. These rhythms are reflected in brain oscillations in the electroencephalogram (EEG). How these rhythms are generated and how rhythmic activity is involved in complex behavior are central questions in neuroscience research, which are still largely unanswered. The research in this thesis is aimed at understanding how synchronized and rhythmic activity is generated in neuronal networks.

Neuronal oscillations have been observed over a wide range of frequencies from 0.05 to 500 Hz (Buzsaki and Draguhn, 2004). They are categorized in frequency bands that are present in the EEG depending on the behavioral state (Figure 1.1). Alpha waves (8-13 Hz) occur more prominently during relaxed wake states, while theta (4-8 Hz) and delta (0.5-4 Hz) band activity occurs especially during different states of sleep (Kandel et al., 2000). Faster oscillations in the beta (13-30) and gamma (30-80 Hz) range are more prominent during active behavior. Rhythms such as alpha, theta and gamma often occur simultaneously and interact with each other (Bragin et al., 1995, Csicsvari et al., 2003, Jensen and Colgin, 2007). Gamma oscillations are observed during active states and are thought to be involved in short-term memory and attention. Gamma oscillations are for example present in humans during working memory tasks (Tallon-Baudry et al., 1998). In several brain disorders there are abnormalities in gamma activity, such as ADHD, schizophrenia, autism, Alzheimer's disease and epilepsy (Herrmann and Demiralp, 2005). Furthermore between healthy individuals there are large variations in oscillations properties as well as in cognitive performance (Vogel, 1981). Thus, it is becoming clear that there are correlations between the occurrence of neuronal network oscillations and cognitive behavior, however to understand their exact relation, one needs to consider three (non-exclusive) aspects of brain oscillations.

Functions of neuronal oscillations

First, brain oscillations in the gamma range may play a role in the temporal binding of information (Gray et al., 1989, Engel et al., 1999, Engel et al., 2001). This theory predicts that neurons that respond to the same sensory object might fire in temporal synchrony with a precision in the millisecond range. In this way information can be stored, processed and

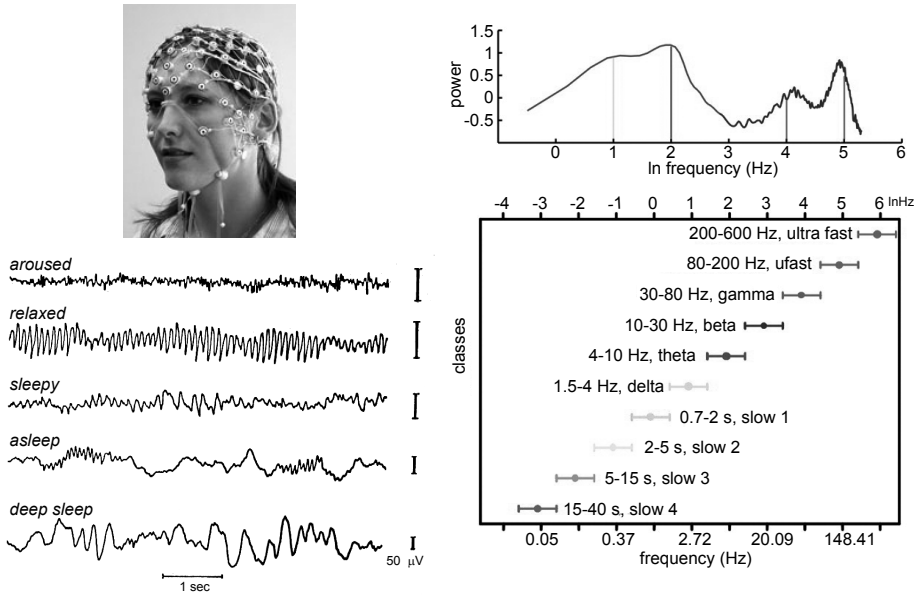

Figure 1.1 EEG activity depends on behavior. (A) person with an EEG cap. (B) EEG activity during different behavioral states. (C) Power spectrum of hippocampal EEG in the mouse. (D) Different EEG bands and their corresponding frequency. (C and D adapted from Buzsaki & Draguhn, 2004).

transferred depending on the specific assembly of neurons that synchronize their firing. For example a neuron that responds to round objects might contribute to the representation of an apple, but might also be involved in the representation of a ball. Neuronal oscillations are thought to enable the synchronization of neurons that correspond to related information. Apart from synchronization within a local region theta and gamma oscillations can also increase the information transfer between brain areas depending of the phase-coupling of the gamma of these regions (Montgomery and Buzsaki, 2007, Sirota et al., 2008, Colgin et al., 2009). In this way the information flow between these two areas might be regulated by the strength of phase-coupling.

A second possible function for network oscillations is that the phase relation of single cell spiking with oscillations can provide information related to time and space (Harris et al., 2002). In the hippocampus it has been shown that when a rat walks through the receptive field of a place neuron, the spiking of this neuron changes relative to theta oscillations (Figure 1.2)(Huxter et al., 2003). In this way information can be stored on the distance the rat has traveled and similar information can be encoded for navigation and temporal order of events. The theta oscillations that are involved in phase precession are superimposed by gamma oscillations (Bragin et al., 1995, Csicsvari et al., 2003). The combination of these two oscillations has been proposed to store short-term memory (Lisman and Idiart, 1995, Lisman, 2005).

Thirdly there might be a role for gamma/theta oscillations in synaptic plasticity

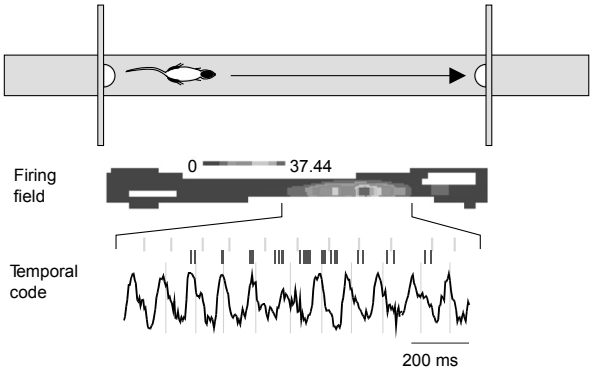

Figure 1.2 Phase of firing during theta depends on place. Firing of a place cell is at a different phase depending of the place in the track. When the rat enters the area where the place cell is active it fires at the peak of the theta wave, while the place cell fires at the through of the theta wave when the rat progresses along the track. Place cell firing of a single run is indicated by red ticks. The False-color plot shows the average firing across multiple runs (Adapted from Huxter et al., 2003).

and memory formation. Processes such as spike-timing-dependent plasticity (STDP) depend on a millisecond time window between pre- and postsynaptic activity, whereby the temporal order of these two can lead to either synaptic potentiation or depression (Bi and Poo, 1998). The time window for STDP of 40 ms falls into the range of beta and gamma activity, and indeed it has been shown that pairing of presynaptic activity with different phases of gamma activity determines the synaptic change (Wespatat et al., 2004).

Since these different possible functions for oscillations are supported by experimental evidence, a next step is to know how different oscillations are generated and how diversity in their occurrence may relate to the specific behavior during which they occur.

What may cause diversity in oscillatory activity?

In humans, individual differences in EEG activity have been described to occur in power and peak frequency of spontaneous or induced oscillations (Posthuma et al., 2001b, Smit et al., 2005). Twin studies suggest that such differences occur because of the high heritability of oscillations (Figure 1.3)(Posthuma et al., 2001b, Smit et al., 2005, Linkenkaer-Hansen et al., 2007) in line with a high heritability of intelligence quotient (IQ) (Dickens and Flynn, 2001, Posthuma et al., 2001b).

Thus, it is likely that genetic variations involved in properties of neuronal oscillations affect cognitive abilities. In line with many studies - but not all - there is a correlation between power, frequency or coherence of alpha, theta and gamma activity and IQ (Vogel, 1981, Doppelmayr et al., 2005, Jausovec and Jausovec, 2005a, b, Benasich et al., 2008), but see also (Posthuma et al., 2001b).

While the heritability of beta and gamma activity is substantial (86% and 49% respectively, Beijsterveldt et al., 1996, Ehlers et al., 2010), only few genes have been

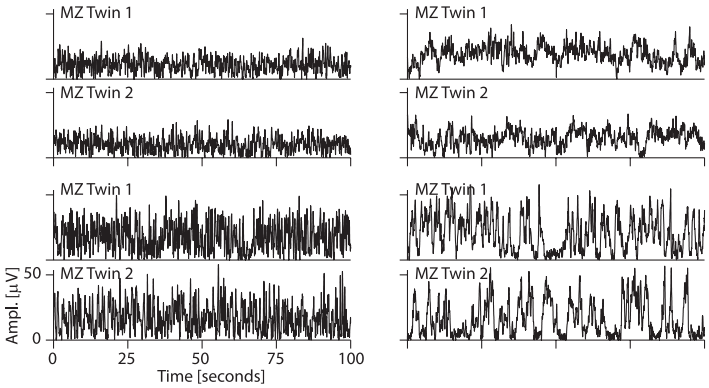

Figure 1.3 Oscillatory activity is highly heritable. Amplitude envelope of alpha waves from monozygotic twins shows a very similar temporal structure, while there are quit large differences within the population. (Adapted from Linkerkear-Hansen et al, 2007).

identified which might underlie differences in oscillations between humans (Deary et al., 2010). Genetic association studies show that a correlation exists between the power in the beta band and a cluster at chromosome 4, which includes the genes for $GABA_A$ receptors α2, α4, and β1 (Porjesz et al., 2002), while polymorphisms in the D4 dopamine receptor and dopamine transporter are correlated with the power in the gamma band (Demiralp et al., 2007). In animal research many other gene products have been shown to be involved in gamma activity in rodents including gap junctions, ionic currents such as Ih, Im, glutamate receptors and $GABA_A$ receptors (Hormuzdi et al., 2001, Fisahn et al., 2002, Mann et al., 2005, Fuchs et al., 2007, Leao et al., 2009), indicating that many additional unknown factors may contribute to the shape and occurrence of neuronal oscillations.

Why study oscillatory network activity in rodent brain?

In general, it is well accepted that polymorphisms which are linked to cognitive performance usually explain only small percentage of the variation within a population and are often hard to reproduce in different populations (Deary et al., 2010). This possibility certainly also holds true for oscillatory activity in the brain, where many factors are involved in properties of neuronal network activity. Hence, the search for a better understanding of the relation between oscillatory activity and cognitive behavior in humans is hampered by the lack of success in finding candidate genes in human association studies.

The study of inbred mouse strains may suffer less from these problems as all mice within a strain are genetically identical, and thereby the statistical power to identify genes involved is much larger. Indeed, using this strategy, several genes have been identified that are involved in anxiety, impulsivity and learning (Wehner et al., 1990, Hovatta et al., 2005, Loos et al., 2009). In addition, animal studies have the advantage that it is possible to specifically target these genes by changing the function or expression levels, which is not possible in human research. Finally the neuronal network that is responsible for the generation of oscillations can be isolated in brain slices, making it easier to directly

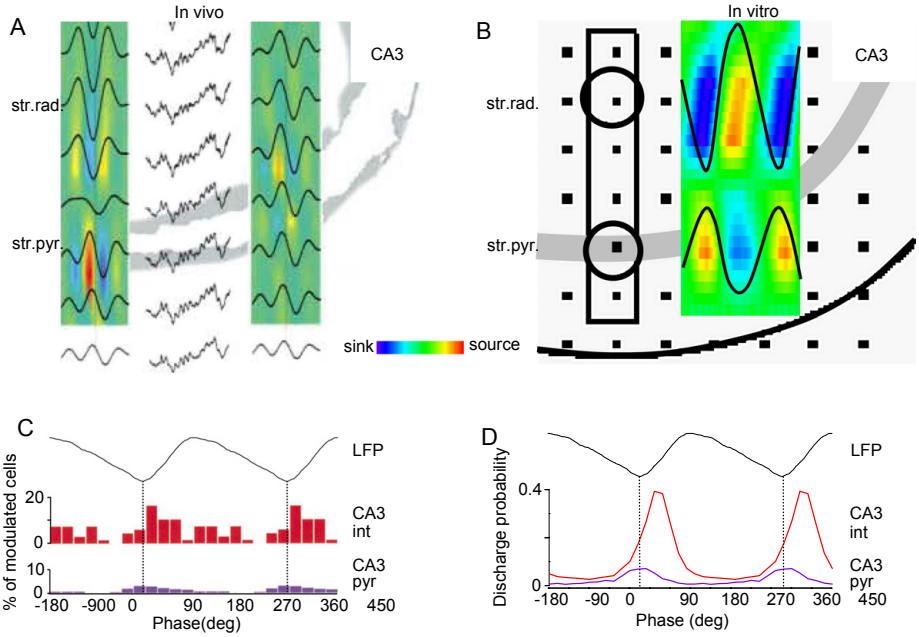

Figure 1. 4 Comparison of gamma oscillations in the hippocampus recorded in vivo with those induced in hippocampal slices by activation of cholinergic receptors. (A, B). In both cases, the field potential reversal is seen between the pyramidal cell layer (str. pyr.) and the apical dendritic layer of pyramidal cells (str. rad.), and the current source density profiles look similar. In addition the spike phase of CA3 pyramidal cells (pyr) and GABAergic interneurons (int) is also equivalent in vivo (C) and in vitro (D), as in both cases, the firing of principal cells at the negative peak of local field potentials (LFP) was followed by the discharge of local inhibitory neurons. (Adapted from Hajos & Paulsen 2009).

manipulate oscillations and relate network activity to single neuron input and behavior.

In vitro models for gamma oscillations

For the experiments in this thesis and in line with the above arguments we have chosen for rodent brain slices in vitro. Slice preparations enable us to study the local network involved in the generation of gamma oscillations without interference of input from other brain regions. As the oscillations in slice models are within the beta/gamma range we refer to them as fast network oscillations. Fast network oscillations can be induced in several different ways, although not all models correspond to gamma oscillation in vivo in terms of neuronal participation and distribution (See Figure 1.4 for a comparison between in vivo oscillations and cholinergicaly-induced oscillations). The best studied models of fast network oscillations in the hippocampus require activation of either muscarinic acetylcholine receptors using for instance carbachol, metabotropic glutamate receptors using DHPG or kainate receptors (Fisahn et al., 1998, Hormuzdi et al., 2001, Palhalmi et al., 2004). In each of these models, oscillations are generated by the CA3 neuronal network

and they depend on GABAergic inhibition (Fisahn et al., 2004, Palhalmi et al., 2004, Mann et al., 2005). A striking difference between kainate and carbachol-induced oscillations is that kainate-induced oscillations are independent of AMPA receptor activation (Fisahn et al., 2004). Kainate-induced oscillations depend on GluR6 receptor activation which increases the excitation of interneurons (Fisahn et al., 2004), while carbachol and DHPG both depolarize CA3 pyramidal cells and thereby indirectly increase the excitation of interneurons (Chuang et al., 2002, Fisahn et al., 2002). On the other hand, interneuron and pyramidal cell firing during oscillations is similar during kainate and carbachol-induced oscillations, indicating that similar networks are involved (Hajos et al., 2004, Gloveli et al., 2005). Carbachol and kainate can also induce oscillations in the neocortex, although they are smaller in power than in the hippocampus, possibly due to the more complex laminar organization of the neocortex (Buhl et al., 1998, van Aerde et al., 2008, van Aerde et al., 2009). Moreover, unlike the hippocampus, where a single rhythm generator exists in CA3, oscillations in the neocortex can be generated independent of each other in different layers and in different areas (Roopun et al., 2006, van Aerde et al., 2008, van Aerde et al., 2009). The frequency of oscillations differs per layer/region, although these oscillations depend on glutematergic and GABAergic inhibition suggesting a similar mechanism as in the hippocampus (Buhl et al., 1998, van Aerde et al., 2008, van Aerde et al., 2009).

Activity of the basal forebrain, which is the major cholinergic input to cortical areas and hippocampus, increases gamma activity in these regions *in vivo*, while gamma activity is absent in the presence of cholinergic blockers (Steriade et al., 1991, Rodriguez et al., 2004). Furthermore the levels of acetylcholine in the brain are related to arousal (Dringenberg and Vanderwolf, 1998).

Therefore, carbachol-induced oscillations in acute brain slices are considered to be an appropriate *in vitro* model of *in vivo* gamma-band oscillations. As the hippocampus has a more compact laminar organization compared to the neocortex, in chapter 2-4, we used this area to study mechanisms involved in the generation of oscillations. For the experiments in chapter 5, where we study how rhythmic input spreads over different layers of a cortical area, visual cortex slices were used.

Perisomatic inhibitory currents drive carbachol-induced oscillations

Carbachol-induced oscillations show an alternating sink/source pair in the stratum pyramidale and stratum radiatum of CA3 in the local field potential (LFP) (Mann et al., 2005). Voltage-sensitive dye imaging showed that currents in the stratum pyramidale are the active events driving the oscillations. Furthermore, local injection of GABA or Glutamate receptor blockers in stratum pyramidale abolishes oscillations showing that synaptic activity in this layer is essential for oscillations. As pyramidal cells fire earlier during oscillations than interneurons, it has been proposed that a local excitatory-inhibitory feedback model is generating the carbachol-induced oscillations (Figure 1.5)(Hajos et al., 2004, Mann et al., 2005). The duration of inhibitory current on pyramidal cells has been thought to determine the spike window of pyramidal cells and thereby the synchronization and frequency of oscillations (Whittington et al., 1995, Traub et al., 2000). Indeed it has been shown that increasing GABAergic kinetics increases the power of oscillations and

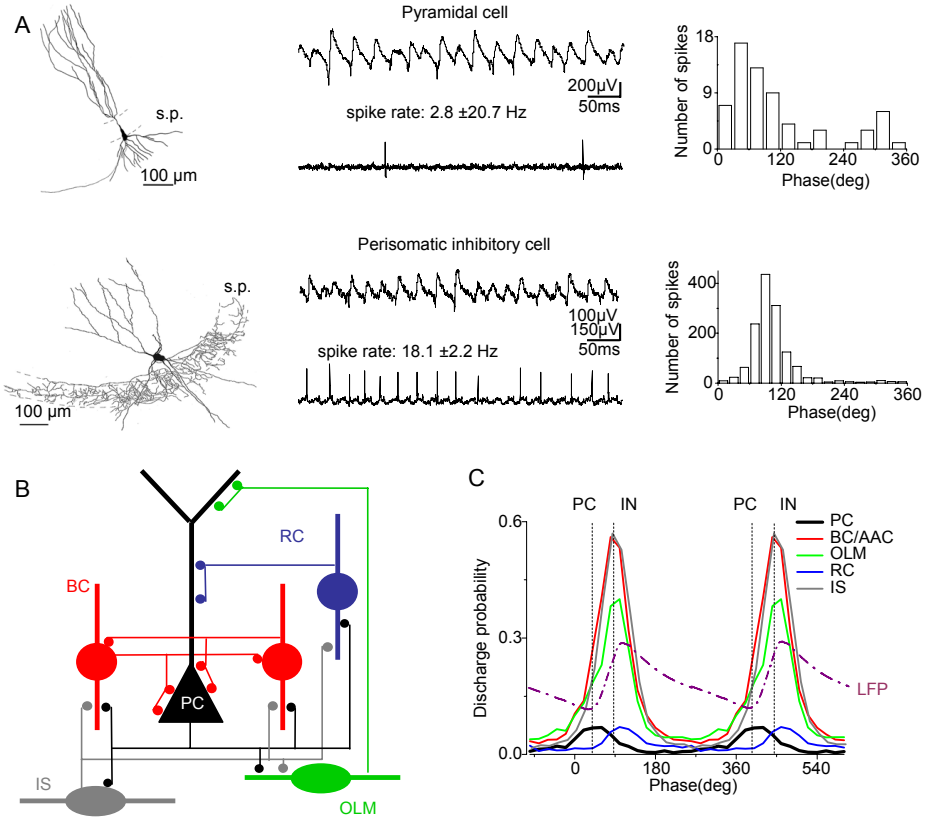

Figure 1.5. Distinct output features of CA3 neurons during gamma oscillations in vitro. (A). Two examples of anatomically-identified cells and their firing properties with the corresponding spike phase histograms during cholinergically-induced gamma oscillations. Pyramidal cells fired at low rate and earlier within a gamma cycle compared to the more active inhibitory neurons. (B). Schematic diagram of the CA3 hippocampal circuitry. (C). Averaged phase histograms of phase-coupled cell types during
gamma oscillations. In all cases, gamma-modulated firing of inhibitory cells (IN) followed the discharge of pyramidal cells (PC). BC, basket cell; AAC, axo-axonic cell; OLM interneurons in the stratum oriens projecting to the stratum lacunosum-moleculare; RC, interneurons with both dendritic and axonal arborizations restricted to the stratum radiatum; IS, cells with horizontal dendritic tree with morphological appearance resembling interneuron-selective interneurons; LFP, local field potential; s.p. stratum pyramidale. (Adapted from Hájos et al. 2004).

decreases the frequency, while increasing AMPA receptor kinetics had no effect (Fisahn et al., 1998, Cope et al., 2005, van Aerde et al., 2009). Furthermore, the amplitude of the inhibitory events determines the duration of the following wave (Atallah and Scanziani, 2009) and the spike probability of interneurons increases prior to changes in the LFP amplitude (Oren et al., 2010a). However, other factors may also influence the frequency

of oscillations, such as the excitability of interneurons or the concentration of the agonist that is used to induce the oscillations (Towers et al., 2004, van Aerde et al., 2009, Mann and Mody, 2010).

Interneuron involvement in gamma oscillations

Since oscillatory activity of neuronal network generated in hippocampus and neocortical areas is dependent both on the activity of neurons as well as interneurons, it is important to note that the interneurons -unlike pyramidal cells, which are thought to be a rather homogenous group of neurons in terms of morphology and function - are much more diverse. Indeed interneurons, although they are all GABAergic, are divided into different classes depending on morphology, firing pattern, expression of proteins involved in cell signaling and on their axonal targets.

Of all interneuron types, basket cells are the best studied interneuron types (Somogyi and Klausberger, 2005). They mainly target the perisomatic region of CA3 pyramidal cells and are highly interconnected. During gamma oscillations in slices they fire at almost every cycle (Figure 1.5)(Hajos et al., 2004, Gloveli et al., 2005). Basket cells are further divided in fast spiking parvalbumin (PV) basket cells and regular spiking cholecystokinin (CCK) interneurons. CCK interneurons express high concentrations of cannabinoid receptors (CB1) receptors (Hajos et al., 2000). Activation of these CB1 receptors reduces the inhibition of CCK cells on pyramidal cells and decreases the amplitude of gamma oscillations (Hajos et al., 2000). Therefore it is likely that CCK cells play a role in the generation of oscillations.

Another interneuron type innervating the perisomatic region of pyramidal cells are axo-axonic or chandelier cells. In their firing pattern they are indiscernible from PV basket cells and they express parvalbumin as well making it difficult to differentiate between these two cell types (Maccaferri et al., 2000). The main difference between these cell types is that axo-axonic cells mainly innervate the axon initial segment of pyramidal cells and can thereby effectively control the firing of pyramidal cells (Somogyi, 1977). There have been some reports claiming that GABAergic currents from axo-axonic cells are excitatory (Szabadics et al., 2006), however recently it was demonstrated that they inhibit the majority of pyramidal cells (Glickfeld et al., 2009).

Finally, experiments using optogenetic techniques, showed that gamma oscillations are reduced in amplitude when parvalbumin cell spiking is prevented (Sohal et al., 2009), whereas driving parvalbumin cells with light flashes between 8 and 200 Hz increased gamma activity (Cardin et al., 2009, Sohal et al., 2009).

Thus both PV and CCK basket cells, but also chandelier cells appear to be actively involved in the generation or modulation of fast network oscillations.

$GABA_A$ receptor diversity

To understand the putative determining role the activity of various types of interneurons may have in generating different activities in oscillatory networks, it is also important to understand the specific functional role of the postsynaptic ionic $GABA_A$ receptors and metabotropic $GABA_B$ receptors. $GABA_A$ receptors are chloride gating channels that

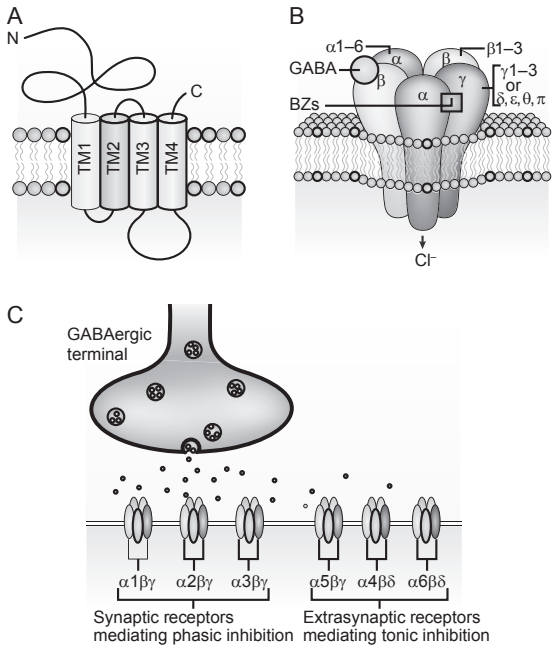

Figure 1.6 GABAA receptor structure and neuronal localization. (A) GABAA receptor subunits consist of four transmembrane domains (TM1–4) (B) Five subunits assemble to form a heteropentameric Cl–-permeable channel. Most GABAA receptors expressed in the brain consist of two α subunits, two β subunits and one γ subunit; the γ subunit can be replaced by δ, ε, θ or π. Binding of the neurotransmitter GABA occurs at the interface between the α and β subunits and triggers the opening of the channel, allowing the rapid influx of Cl– into the cell. (C) GABAARs composed of α (1–3) subunits together with β and γ subunits are thought to be primarily synaptically localized, whereas α5βγ receptors and α(4 or 6)βδ are localized at extrasynaptic sites. (Adapted from Jacob et al., 2008).

belong to the superfamily of ligand-gated ion channels and are the most common receptors in the brain involved in synaptic inhibition. $GABA_A$ receptors form pentameric complexes assembled from at least 12 subunits in the neocortex and hippocampus: α1-5, β1-3, γ1-3 and δ. The heterogeneity is much larger than that of other ligand-gated ion channels and suggests a wide diversity of functionality and distribution of the receptor. Most $GABA_A$ receptors are composed of two α, two β, and one γ or δ (Figure 1.6), with α1β2γ2 being the most common and represent 60% of the entire population, followed by α2β3γ2 (Mohler, 2006). Especially the α subunit is thought to be essential for the kinetics and sub-cellular localization of the $GABA_A$ receptor (Banks and Pearce, 2000, Hutcheon et al., 2000, Nyiri et al., 2001, Vicini et al., 2001, Klausberger et al., 2002, Bosman et al., 2005b). The α1 subunit is expressed both extra-synaptically and synapically (Thomas et al., 2005) and is responsible for fast IPSC decay time kinetics (Vicini et al., 2001, Bosman et al., 2005b). Especially synapses from PV basket cells in CA1 contain α1 subunits (Figure 1.7), but also bistratified cells express α1 subunits (Klausberger et al., 2002). The α2 subunit is located at synapses from CCK basket cells and axo-axonic cells in CA1 (Figure 1.7)(Nyiri et al., 2001) and has slower decay time kinetics than α1 containing receptors (Brussaard et al., 1997, Lavoie et al., 1997). The sub-cellular location of the α3 subunit is less well studied, although they are expressed at the axon initial segment of layer 5 pyramidal cells (Fritschy et al., 1998) and have similar decay time kinetics as the α2 subunit (Verdoorn, 1994). $GABA_A$ receptors with α4 subunits are thought to be co-localized with the δ subunit and are located extra-synaptically in interneurons (Nusser et al., 1998, Korpi et

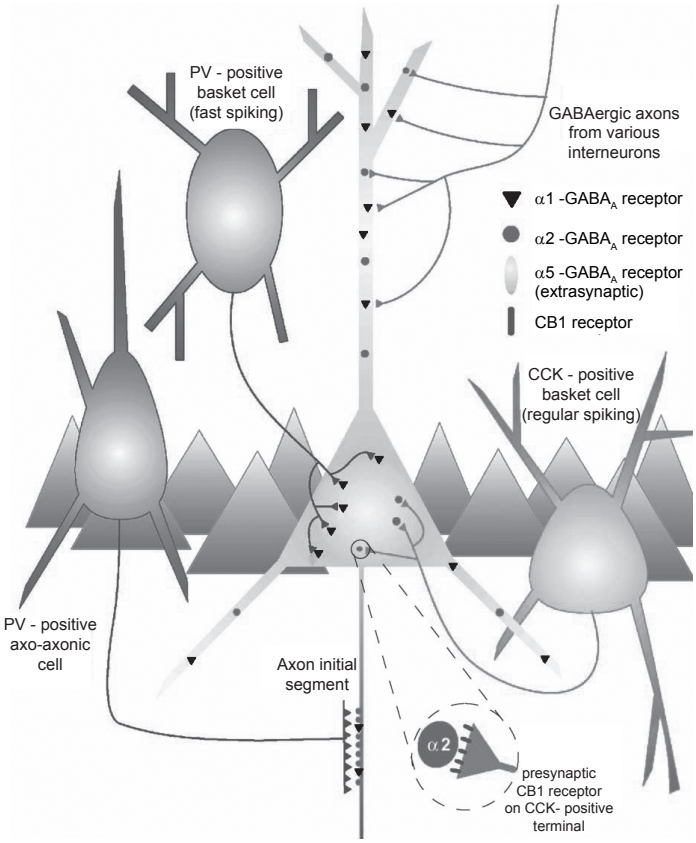

Figure 1.7 Subcellular distribution of GABAA receptor subtypes in relation to synaptic inputs in a CA1 hippocampal pyramidal cell. A segregation of α1- and α 2-GABAA receptor clusters is evident on the soma and axon initial segment according to different populations of GABAergic interneurons. Extrasynaptic α 5-GABAA receptors are distributed on the entire somato-dendritic compartment. CCK, cholecystokinin; PV, parvalbumin. (Adapted from Fritschy & Brünig, 2003).

al., 2002, Peng et al., 2002). The α5 subunit is mainly expressed in the hippocampus and is localized both extra-synaptic and at distal sites and is thought to mediate $GABA_{A,slow}$ (Fritschy and Brunig, 2003, Prenosil et al., 2006). So which $GABA_A$ receptor subunits mediate the feedback inhibition required for fast network oscillations? The answer to this question might give more insight in which synapses and interneuron types are involved in fast network oscillations and how $GABA_A$ receptor kinetics and location determine the synchronization and frequency of hippocampal network oscillations.

Aims of this thesis

As described above, fast network oscillations may be highly diverse between individuals and depending on their cognitive behavior taking place at any given moment. Hence,

mechanisms modulating oscillations might have huge impact on cognitive performance. Therefore, we aim to identify factors involved in the generation, modulation and spread of rhythmic activity, with a focus on feedback inhibition from interneurons and activation of postsynaptic GABAergic receptors.

To reach this aim we used both hypothesis free approaches to find new genes and proteins, as well as targeted studies at $GABA_A$ receptor involvement. In doing so the following research questions were addressed:

1. Are there differences between various genetically distinct so-called *common inbred* mouse strains in properties of carbachol-induced oscillations in the hippocampus that can point to genetic factors that can explain variation in oscillation properties between mouse strains? In chapter 2 we compared the properties of carbachol induced oscillations in eight inbred mouse strains. In this hypothesis free approach the heritability of more than 200 quantitative traits is derived from this oscillatory activity.

2. What mechanism might underlie the differences in oscillations between common inbred mouse strains? As the local field potential is shaped by synaptic activity, we studied synaptic mechanisms which might explain the differences in oscillations between inbred mouse strains observed in chapter 2. In chapter 3 we combined single cell with multi electrode recordings to find out whether diversity in spiking or synaptic activity might underlie differences in rhythmic activity.

3. Which $GABA_A$ receptor α subunits are involved in carbachol induced oscillations, and which synapses set the frequency in the hippocampus? We addressed this question in chapter 4 by using mutant mice which are either lacking a $GABA_A$ receptor α -subunit or have a point mutation in one of the α -subunits which makes their $GABA_A$ receptors insensitive to allosteric modulation at the benzodiazepine binding site. We combined multi electrode recordings with single cell recordings to find out which α subunit is responsible for the perisomatic feedback inhibition on pyramidal cells.

4. Does GABAergic inhibition influence rhythmic input into the visual cortex? During the processing of sensory information the visual cortex receives rhythmic input from several brain areas including the thalamus (Llinas and Steriade, 2006). In chapter 5 we stimulated the incoming fibers and monitored the spreading of the different layers using voltage sensitive dye imaging. By allosteric modulation of $GABA_A$ receptor we studied the role of inhibition in the spreading of rhythmic input.